A new species of the odorous frog genus *Odorrana* (Amphibia, Anura, Ranidae) from southwestern China

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ABSTRACT

The genus *Odorrana* is widely distributed in the mountains of East and Southeastern Asia. An increasing

22 number of new species in the genus have been recognized especially in the last decade. Phylogenetic studies of

23 the *O. schmackeri* species complex with wide distributional range also revealed several cryptic species. Here,

24 we describe a new species in the species complex from Guizhou Province of China. Phylogenetic analyses

25 based on mitochondrial DNA indicated the new species as a monophyly clustered into the *Odorrana* clade and

26 sister to *O. schmackeri*, and nuclear DNA also indicated it as an independent lineage separated from its related

27 species. Morphologically, the new species can be distinguished from its congeners based on a combination of

28 the following characters: (1) having smaller body size in males (snout-vent length < 43.3 mm); (2) head longer

29 than wide; (3) dorsolateral folds absent; (4) tympanum of males large and distinct, tympanum diameter twice

30 as long as width of distal phalanx of finger III; (5) two metacarpal tubercles; (6) relative finger lengths: II < I <

31 IV < III; (7) tibio-tarsal articulation reaching to the level between eye to nostril when leg stretched forward; (8)

32 disks on digits with circum-marginal grooves; (9) toes fully webbed to disks; (10) the first subarticular tubercle

33 on fingers weak; (11) having white pectoral spinules, paired subgular vocal sacs located at corners of throat,

34 light yellow nuptial pad on the first finger in males.

40 **INTRODUCTION**

41 Frogs of the genus *Odorrana* Fei, Ye & Huang, 1990 inhabit montane streams in the subtropical and

42 tropical regions of East and Southeast Asia, ranging from the Ryukyu Archipelago of southern Japan, southern

43   China and Indochina, northeastern India, Myanmar and Malay Peninsula to Sumatra and Borneo (Frost, 2018).

44   Although the systematic relationships of the group had been controversial for decades, it has been recognized

45   as a monophyly in recent years (e.g. Matsui et al., 2005; Ngo et al., 2006; Cai et al., 2007; Che et al., 2007;

46   Stuart, 2008; Wiens et al., 2009; Pyron & Wiens, 2011; Chen et al., 2013). The genus currently contains 58

47   recognized species (Frost, 2018). Noticeably, in the last decade, 13 species have been described (Li et al., 2008;

48   Tran et al., 2008; Yang, 2008; Bain et al., 2009; Chen et al., 2010a, b; Kuramoto et al., 2011; Mo et al., 2015;

49   Wang et al., 2015; Pham et al., 2016; Saikia et al., 2017). This indicated that the species diversity has been

50   underestimated and more discoveries were obligatory in the genus.

51   The piebald odorous frog *O. schmackeri* was firstly found in Gaojiayan Town, Changyang County (Co.),

52   Hubei Province (Prov.), China, and named by Boettger (1892) only based on one male specimen and with a

53   negligible description. Thus, Liu & Hu (1961) redescribed *O. schmackeri* based on some specimens from Daba

54   Mountains and Xiushan Co. in Chongqing City of China near to the type locality of the species, and presented

55   the diagnosis characters for the species, such as having great circle brown spots on dorsa and the outer vocal

56   sac below pharynx in males. Afterwards, based on mass of reports and investigations, the species has been

57   suggested to be distributed in Henan, Shaanxi, Gansu, Sichuan, Guizhou, Hubei, Anhui, Jiangsu, Zhejiang,

58   Hunan, Guangdong and Guangxi provinces of China (Fei et al., 2009, 2012), even northern Thailand (Chan-

59   ard, 2003) and northern Vietnam (Orlov et al., 2002; Nguyen et al., 2005). This group is highly adapted to

60   mountain environments, inhabiting the moist evergreen broad-leaf forests and streams at 200-1400 m altitudes

61   (Fei et al., 2009). Accordingly, it could be assumed that the wide distributional range and strict habitat

62   requirements might promote considerable divergence even speciation due to isolation in the group.

63   Correspondingly, populations ever classified as *O. schmackeri* were suggested to be much variable in

64   morphology (Fei et al., 2009, 2012), indicating that there might be cryptic species in the group. Indeed, from

65   this group, several new species have been constantly described: *O. nanjiangensis* Fei, Ye, and Jiang, 2007

66   occurring from Nanjiang County (Co.), Sichuan Province (Prov.); *O. huanggangensis* Chen, Zhou and Zheng,

67   2010a occurring from Wuyi Mountains in Fujian and Jiangxi provinces; and *O. tianmuii* Chen, Zhou and

68   Zheng, 2010b occurring from Tianmu Mountains in Zhejiang Prov.. However, phylogenetic analyses of Chen

69   et al. (2013) indicated that above four species did not form a monophyly, but were nested with some other

70   *Odorrana* species including two cryptic species, and also strongly rejected the alliance of *O. anlungensis*, *O.*

71   *yizhangensis* and *O. lungshengensis* with the *O. schmacheri* group that was defined based on morphological

72   data (Fei et al., 2009). So the "*O. schmackeri* species complex" was defined referring to the species resembling

73   *O. schmackeri* on morphology, such as *O. nanjiangensis*, *O. huanggangensis*, *O.hejiangensis*, *O. tianmuii* and

74   several cryptic species (e.g. Li et al., 2015; Zhu, 2016; He, 2017).

75   Recently, many studies have paid attention to the phylogenetic relationships and diversification of the *O.*

76   *schmackeri* species complex (e.g. Li et al., 2015; Zhu, 2016). Li et al. (2015) presented a phylogeographic

77   framework for the species complex using 25 populations based on mitochondrial ND2 and two tRNA gene

78   sequences, and proposed seven clades: Clade A was (*O. huanggangensis* + "*O. yizhangensis*" identified by

79   them) from Wuyi Mountains in Fujian Prov., Nanling Mountains in the border of Guangdong and Hunan

80   provinces and mountains in eastern Guizhou Prov.; Clade B was *O. tianmuii* occurring from Huangshan

81   Mountains in Anhui Prov. and Tianmu Mountains in Zhejiang Prov.; Clade C was *O. schmackeri sensu stricto*

82   occupying a more narrow distribution area in Funiu Mountains in Henan and northern Hunan provinces and

83   Daba Mountains in Hubei Prov.; Clade D was proposed as a cryptic species from mountains in Jiangxi Prov.;

84   Clade E was the second cryptic species from mountains of northwestern Guizhou Prov.; Clade F was the third

85   cryptic species from Funiu Mountains in Henan Prov. and being sympatric with Clade C; and Clade G was the

86   fourth cryptic species from Daba Mountains in Hubei Prov. and also being sympatric with *O. schmackeri sensu*

87   *stricto* (Fig. 1). As well, Zhu (2016) based on 12S rRNA and 16S rRNA genes showed a broadly similar

88   phylogeographic framework through a more comprehensive sampling with 78 populations, and supplied

89   several different implications: Clade D in Li et al. (2015) should not be recognized as a cryptic species but still

90   be classified as *O. schmackeri*, and thus *O. schmackeri sensu stricto* was in fact distributed in western Henan,

91   southeastern Shanan'xi, eastern to central Chongqing, northwestern and eastern Hubei, northwestern Hunan,

92   northeastern Guizhou and Jiangxi provinces in China; *O. hejiangensis* occupied a large range around Sichuan

93   Basin even in western Henan and eastern Shananxi provinces; one cryptic species (*Odorrana* sp1 defined by

94   them) had a large range across central Guizhou and Guangxi provinces; and another cryptic species (*Odorrana*

95   sp2 defined by them) occupied a large range around the southern part of Sichuan Basin (Fig. 1). However,

96   because of different samplings and use of different genes in the two studies especially Li et al. (2015) having

97   no morphological data and no releasing of sequences in Zhu (2016), there were still several uncertain points: (1)

98   whether Clade E in Li et al. (2015) containing only two populations (Jinsha Co. and Suiyang Co. in Guizhou

99   Prov.) belonged to *Odorrana* sp1 in Zhu (2016); (2) whether Clade F (the third cryptic species) from Funiu

100   Mountains in Henan Prov. in Li et al. (2015) belonged to *O. hejiangensis*; (3) whether Clade G from Daba

101   Mountains in Li et al. (2015) belonged to *Odorrana* sp2 in Zhu (2016). Anyway, several cryptic species in the

102   species complex have been indicated. To better understand diversification of the species complex, it is

103   necessary and urgent to make more investigations (e.g. distribution range, morphology, molecular

104   phylogenetics, ecology and tadpoles) on the new taxa.

105   From 2013 to 2017, we carried out a series of biodiversity surveys in Guizhou Prov. of China and

106 collected many specimens of *Odorrana* superficially resembling *O. schmackeri*. As note, some of them were

107 collected from the population in the Lengshuihe Reserve in Jinsha Co., Guizhou Prov., China (Fig. 1) where

108 the samples of Clade E in Li et al. (2015) indicated as a cryptic species was collected. To distinguish these

109 specimens, we conducted phylogenetic analyses based on mitochondrial DNA and nuclear DNA and

110 morphological comparisons. All analyses consistently supported our specimens from Guizhou Prov. as a new

111 taxon. Hence, we describe it herein as a new species.

112

113 **METHODS**

114 **Sampling**

115  Frogs were collected on September 17 and 21, 2013 and August 3, 4 and 8, 2015, and tadpoles were

116 collected on October 10, 2017. After taking photographs, they were euthanized using isoflurane, and then the

117 specimens were fixed in 10% buffered formalin. Tissue samples were taken and preserved separately in 95%

118 ethanol prior to fixation. Specimens collected in this work were deposited in Chengdu Institute of Biology,

119 Chinese Academy of Sciences (CIB, CAS).

120  A total of 25 adult specimens of the new taxon including 16 females and nine males were collected from

121 the mountain streams of three localities across Guizhou Province of China (Fig. 1; for voucher information see

122 Table S1). For comparisons, some specimens of the related species were also collected in this study, including

123 19 *O. schmackeri* specimens from two localities, 23 *O. huanggangensis* specimens from three localities, three

124 *O. yizhangensis* specimens from two localities and two *O. lungshengensis* specimens from one locality (Fig. 1;

125 for voucher information see Table S1).

126  Ten tadpoles with almost identical morphology were collected from the same place in the stream where

127   the new taxon was found in the Lengshuihe Nature Reserve of Jinsha Co., Guizhou Prov., China. They were

128   identified as the new taxon because they were almost identical in morphology and one representative of them

129   was genetically close to the adult specimens of the new taxon (see the results). Stages of tadpoles were

130   identified following Gosner (1960).

131   **Collection of molecular data**

132   A total of 18 molecular samples were collected in this study: five containing four adults and one tadpole of

133   the new taxon, five of *O. schmackeri* including two topotypes, three of *O. huanggangensis*, three of *O.*

134   *yizhangensis* including one topotype, and two of *O. lungshengensis* (for voucher information see Table S2).

135   Total DNA was extracted using a standard phenol-chloroform extraction protocol (Sambrook et al., 1989).

136   The mitochondrial 12S rRNA, 16S rRNA and ND2 genes and two nuclear protein-coding genes (DOLK and

137   KCNF genes) were amplified and sequenced from our samples. Primer sequences were acquired from

138   literatures for 12S rRNA (Kocher et al., 1989), 16S rRNA (Simon et al., 1994), ND2 (Li et al., 2015), DOLK

139   (Shen et al., 2013) and KCNF (Shen et al., 2013) genes (primer sequences were presented in Table S3). PCR

140   amplification reactions for mitochondrial genes were performed in a 30 μL volume reaction with the following

141   cycling conditions: an initial denaturing step at 95 °C for 4 min; 36 cycles of denaturing at 95 °C for 40 s,

142   annealing at 55 °C (for 12S and 16S)/47 °C-57 °C (for ND2) for 40 s and extending at 72 °C for 70 s, and a

143   final extending step of 72 °C for 10 min. Amplifications of nuclear genes were according to Shen *et al.* (2013).

144   PCR products were purified with spin columns and then were sequenced with both forward and reverse

145   primers same as used in PCR. Sequencing was conducted using an ABI3730 automated DNA sequencer in

146   Shanghai DNA BioTechnologies Co., Ltd. (Shanghai, China). All new sequences were deposited in GenBank

147   (for GenBank Accession numbers see Table S2).

148   For phylogenetic analyses , we downloaded 12S and 16S gene sequences from GenBank for all those

149   related species especially for their topotypes for which comparable sequences were available (for GenBank

150   Accession numbers see Table S2) based on the previous studies (Chen *et al*., 2013; Li *et al*., 2015). As note,

151   ND2 gene sequences for most *Odorrana* species were not sequenced to now, impeding us to examine

152   comprehensive relationships of the genus using this gene. To further understand the divergence between the

153   new taxon and its related species, ND2 sequences for all haplotypes of the *O. schmackeri* species complex in

154   Li et al. (2015) were downloaded (for GenBank Accession number see Table S2).

155   **Phylogenetic analyses and genetic distance estimation**

156   Sequences were assembled and aligned using the ClustalW module in BioEdit v. 7.0.9.0 (Hall, 1999) with

157   default settings. The datasets were checked by eye and revised manually if necessary. To avoid bias in

158   alignments, GBLOCKS v. 0.91.b (Castresana, 2000) with default settings was used to extract regions of

159   defined sequence conservation from the length-variable 12S and 16S fragments. No-sequenced fragments were

160   treated as missing data. At last, for phylogenetic analyses of mitochondrial DNA, two datasets were obtained,

161   i.e. three-genes concatenated dataset with 12S (for 61 samples) + 16S (for 61 samples) + ND2 (for 16 samples)

162   and ND2 gene alone dataset (for 112 samples).

163   Based on the three-genes concatenated dataset, phylogenetic analyses were conducted using maximum

164   likelihood (ML) and Bayesian Inference (BI) methods, implemented in PhyML v. 3.0 (Guindon *et al.*, 2010)

165   and MrBayes v. 3.12 (Ronquist & Huelsenbeck, 2003), respectively. One *Rana chensinensis* was chosen as

166   outgroup according to the previous studies (Pyron & Wiens, 2011; Chen et al., 2013). To avoid under- or over-

167   parameterization (Lemmon & Moriarty, 2004; McGuire et al., 2007), for the phylogenetic analyses, the best

168   partition scheme and the best evolutionary model for each partition were chosen using PARTITIONFINDER v.

169   1.1.1 (Lanfear et al., 2012). In this analysis, 12S, 16S genes and each codon position of ND2 gene were

170   defined and Bayesian Inference Criteria (BIC) was used. As a result, the analysis suggested that the best

171   partition scheme is 12S gene/16S gene/each codon position of ND2 gene, and selected TrN+I+G model as the

172   best model for 12S, 16S and the second codon position of ND2 gene and GTR+I+G model as the best model

173   for the other two codon position of ND2 gene. For the ML tree, branch supports were drawn from 10000 non-

174   parametric bootstrap replicates. In BI analyses, the parameters for each partition were unlinked, and branch

175   lengths were allowed to vary proportionately across partitions. Two runs each with four Markov chains were

176   simultaneously run for 50 million generations with sampling every 1000 generations. The first 25% trees were

177   removed as the "burn-in" stage followed by calculations of Bayesian posterior probabilities and the 50%

178   majority-rule consensus of the post burn-in trees sampled at stationarity.

179   To further visualize the degree of genetic splits among the new taxon and its related species especially

180   members of the *O. schmackeri* species complex recognized by Li et al. (2015), a phylogenetic network using

181   the maximum parsimony method in Splittree v. 4.11.3 (Huson & Bryant, 2006) was constructed based on the

182   ND2 gene sequence dataset. The supports of Splittree lineages were evaluated by 1000 bootstrap replicates.

183   In addition, to access the genetic divergence between the new taxon and its related species on nuclear

184   DNA, haplotype networks for DOLK and KCNF gene datasets were constructed, respectively, using the

185   maximum parsimony method in TCS v. 1.21 (Clement et al., 2000).

186   Finally, pairwise uncorrected *p*-distance on the 16S rRNA gene were estimated using MEGA v. 6.06

187   (Tamura *et al.*, 2011) to evaluate the genetic divergence between *Odorrana* species.

188   **Morphological comparisons**

189   A total of 67 adult specimens including nine males and 16 females of the new taxon, 15 males and four

190   females of *O. schmackeri* and 13 males and ten females of *O. huanggangensis* were measured (for voucher

191   information see Table S1). Ten tadpoles of the new taxon were measured (for voucher information see Table

192   S4). The terminology and methods followed Fei et al. (2009). Measurements were taken with a dial caliper to

193   the nearest 0.1 mm. Twenty one morphometric characters of adult specimens were measured: SVL = snout-

194   vent length (distance from the tip of the snout to the posterior edge of the vent), HDL = head length (distance

195   from the tip of the snout to the articulation of jaw), HDW = maximum head width (greatest width between the

196   left and right articulations of jaw), SL = snout length (distance from the tip of the snout to the anterior corner

197   of the eye), ED = eye diameter (distance from the anterior corner to the posterior corner of the eye), IOD =

198   interorbital distance (minimum distance between the inner edges of the upper eyelids), IND = internasal

199   distance (minimum distance between the inner margins of the external nares), NED = nasal to eye distance

200   (distance between the nasal and the anterior corner of the eye), NSD = nasal to snout distance (distance

201   between the nasal the posterior edge of the vent), IFE = distance between anterior corner of eyes, IAE =

202   distance between posterior corner of eyes, TYD = maximal tympanum diameter, LAL = length of lower arm

203   and hand (distance from the elbow to the distal end of the Finger IV), LW = lower arm width (maximum width

204   of the lower arm), THL = thigh length (distance from vent to knee), TL = tibia length (distance from knee to

205   tarsus), TW = maximal tibia width, TFL = length of foot and tarsus (distance from the tibiotarsal articulation to

206   the distal end of the Toe IV), FL = foot length (distance from tarsus to the tip of fourth toe), FDW = finger disk

207   width (width at the widest part of the disk of finger III), DPW = distal phalanx width (maximal width of distal

208   phalanx of finger III). Ten morphometric characters of larvae were measured: TOL = total length, SVL =

209   snout-vent length, BH = maximum body height, BW = maximum body width, SL = snout length (distance

210   from the anterior corner of the eye to the tip of the snout), SS = snout to spiraculum (distance from spiraculum

211   to the tip of the snout), MW = mouth width (distance between two corners of mouth), TBW = maximum width

212   of tail base, TL = tail length (distance from base of vent to the tip of tail), TH = tail height (maximum height

213   between upper and lower edges of tail).

214   In order to reduce the impact of allometry, the correct value from the ratio of each measurement to SVL

215   was calculated and then log-transformed for the following morphometric analyses. One-way analysis of

216   variance (ANOVA) was used to test the significance of differences on morphometric characters between males

217   and females and between different species. The significance level was set at 0.05. To show the spatial

218   distribution of different species on the morphometric characters, principal component analyses (PCA) were

219   performed. These analyses were carried out in the R (R Development Core Team, 2008).

220   The new species was also compared with all other *Odorrana* species on morphology. Comparative

221   morphological data were obtained from literatures for *O. absita* (Stuart & Chan-ard, 2005), *O. amamiensis*

222   (Matsui, 1994), *O. andersonii* (Boulenger, 1882), *O. anlungensis* (Hu et al., 1973), *O. arunachalensis* (Saikia

223   et al., 2017), *O. aureola* (Stuart et al., 2006), *O. bacboensis* (Bain *et al.*, 2003), *O. banaorum* (Bain et al.,

224   2003), *O. bolavensis* (Stuart & Bain, 2005), *O. cangyuanensis* (Yang, 2008), *O. chapaensis* (Bourret, 1937), *O.*

225   *chloronota* (Günther, 1876), *O. exiliversabilis* (Li et al., 2001 in Fei et al., 2001b), *O. fengkaiensis* (Wang *et al.*,

226   2015), *O. geminata* (Bain et al., 2009), *O. gigatympana* (Orlov et al., 2006), *O. grahami* (Boulenger, 1917), *O.*

227   *graminea* (Boulenger, 1900), *O. hainanensis* (Fei et al., 2001a), *O. hejiangensis* (Deng &Yu, 1992), *O. hosii*

228   (Boulenger, 1891), *O. huanggangensis* (Chen et al., 2010a), *O. heatwolei* (Stuart & Bain, 2005), *O.*

229   *hmongorum* (Bain *et al.,* 2003), *O. indeprensa* (Bain & Stuart, 2005), *O. ishikawae* (Stejneger, 1901), *O.*

230   *jingdongensis* (Fei et al., 2001a), *O. junlianensis* (Fei & Ye, 2001 in Ye and Fei, 2001), *O. khalam* (Stuart et

231   al., 2005), *O. kuangwuensis* (Liu and Hu, 1966 in Hu et al., 1966), *O. leporipes* (Werner, 1930), *O.*

232   *lipuensis* (Mo *et al.*, 2015), *O livida* (Blyth, 1856), *O. macrotympana* (Yang, 2008), *O. margaretae* (Liu, 1950),

233   *O. mawphlangensis* (Pillai & Chanda, 1977), *O. monjerai* (Matsui & Jaafar, 2005), *O. morafkai* (Bain et al.,

234   2003), *O. mutschmanni* (Pham *et al*., 2016), *O. nanjiangensis* (Fei et al., 2007a), *O. narina* (Stejneger, 1901),

235   *O. nasica* (Boulenger, 1903), *O. nasuta* (Li et al., 2001 in Fei et al., 2001b), *O. orba* (Stuart & Bain, 2005), *O.*

236   *rotodora* (Yang & Rao, 2008 in Yang, 2008), *O. schmackeri* (Boettger, 1892), *O. sinica* (Ahl, 1927), *O.*

237   *splendida* (Kuramoto et al., 2011), *O. supranarina* (Matsui, 1994), *O. swinhoana* (Boulenger, 1903), *O.*

238   *tianmuii* (Chen et al., 2010b), *O. tiannanensis* (Yang & Li, 1980), *O. tormota* (Wu, 1977), *O. trankieni* (Orlov

239   et al., 2003), *O. utsunomiyaorum* (Matsui, 1994), *O. versabilis* (Liu & Hu, 1962), *O. wuchuanensis* (Xu, 1983

240   in Wu et al., 1983), *O. yentuensis* (Tran et al., 2008), *O. yizhangensis* (Fei et al., 2007b) and *O. zhaoi* (Li et al.,

241   2008).

242   **Skull scanning**

243   Skulls of two male specimens (voucher number: CIBjs20150803001, CIBjs20150803002) and three

244   female specimens (voucher number: CIBjs20150804001, CIBGYU20130917003, CIBGYU20130917001) of

245   the new taxon were scanned in the high-resolution X-ray scanner (Quantum GX micro-CT Imaging System,

246   PerkinElmer®). The specimens were scanned along the coronal axis at an image resolution of 2000 * 2000.

247   Segmentation and three-dimensional reconstruction of the CT images were made using VG57 Studio Max 2.2

248   (Volume Graphics, Heidelberg, Germany).

249   The Animal Care and Use Committee of Chengdu Institute of Biology, CAS provided full approval for

250   this purely observational research (Number: CIB2013041102). Field work was approved by the Management

251   Office of the Lengshuihe Nature Reserve (project number: LSH-201304003).

252   The electronic version of this article in Portable Document Format (PDF) will represent a published work

253   according to the International Commission on Zoological Nomenclature (ICZN), and hence the new names

254   contained in the electronic version are effectively published under that Code from the electronic edition alone.

255   This published work and the nomenclatural acts it contains have been registered in ZooBank, the online

256   registration system for the ICZN. The ZooBank LSIDs (Life Science Identifiers) can be resolved and the

257   associated information viewed through any standard web browser by appending the LSID to the prefix

258   http://zoobank.org/. The LSID for this publication is: urn:lsid:zoobank.org:pub:E98B65CB-E8E3-4412-9613-

259   D9DD32A77B99. The online version of this work is archived and available from the following digital

260   repositories: PeerJ, PubMed Central and CLOCKSS.

261

262   **RESULTS**

263   **Phylogenetic analyses and genetic divergence**

264   Aligned sequence matrix of 12S+16S, ND2, DOLK and KCNF genes contained 1835 bp, 768 bp, 645 bp

265   and 750 bp, respectively. ML and BI analyses based on the 12S+16S+ND2 matrix resulted in essentially

266   identical topologies with high node supporting values (Fig. 2A). All samples of the new taxon occurring from

267   Guizhou Prov. were strongly clustered into a monophyly, which was placed into the genus *Odorrana* and sister

268   to the *O. schmackeri* clade. ND2 splitstree also strongly supported the splits between the new taxon and its

269   related species (Fig. 2B). As note, *Odorrana* sp2 simultaneously revealed by Li et al. (2015) and Zhu (2016)

270   and *Odorrana* sp3 revealed by Li et al. (2015) also occupied an independent lineage, respectively. Only one

271   haplotype was found for all samples of the new taxon, either in KCNF gene or in DOLK gene, and there was

272   no common haplotype between the new species and its related species (Fig. 3).

273   The genetic distances on 16S gene between the new taxon and its sister species *O. schmackeri* was mean

274   0.027 (range 0.026–0.028; Table S5), much higher than the intraspecific genetic distance within each of the

275   new taxon, *O. schmackeri*, *O. huanggangensis*, *O. yizhangensis* and *O. lungshengensis* (all intraspecific

276   genetic distance < 0.002). More significantly, it was higher or even much higher than the interspecific genetic

277   distance between many sister species, for example, that between *O. huanggangensis* and *O. tianmuii* (0.014),

278   *O. hainanensis* and *O. fengkaiensis* (0.013), *O. nasuta* and *O. versabilis* (0.013), *O. versabilis* and *O.*

279   *exiliversabilis* (0.017), *O. morafkai* and *O. banaorum* (0.013), and *O. grahami* and *O. jingdongensis* (0.017;

280   Table S5).

281   **Morphological comparisons**

282   The results of one-way ANOVA showed that in the new taxon, the males was significantly different from

283   the females on SVL and the ratios of ED, IND, IFE, LAL, TYD, LW and TW to SVL (all p-values < 0.05;

284   Table 1). So, morphometric analyses were conducted on males and females, respectively. In PCA for males,

285   the total variation of the first two principal components was 42.23%, and in PCA for females, it is 37.37%. In

286   both males (Fig. 4A) and females (Fig. 4B), on the two-dimensional plots of PC1 versus PC2, the new taxon

287   could be almost separated from *O. schmackeri*. The results of one-way ANOVA indicated that either in males

288   or females, the new taxon was significantly different from *O. schmackeri* and *O. huanggangensis* on many

289   morphometric characters (all p-values < 0.05; Tables 1). More detailed descriptions of results from

290   morphological comparisons between the new taxon and its congeners were presented in the following sections

291   for describing the new species.

292   At all, molecular and morphological results supported that our specimens from Guizhou Prov. of China

293   was a new taxon. It is described as a new species in the following sections:

294   ***Odorrana kweichowensis* sp. nov.**

295   urn:lsid:zoobank.org:act:95123118-30D2-42B5-B5DB-AB7C32B33F97

296   **Holotype**

297   CIBjs20150803002, adult male (Fig. 5A and B; Fig. 6A and B), collected by S.Z. Li in the Lengshuihe Nature

298   Reserve (27.47361°N, 106.00139°E; elevation 754 m a.s.l.), Jinsha Co., Guizhou Prov., China.

299   **Paratypes**

300   24 specimens (nine adult males and 15 adult females), 14 specimens collected by S.Z. Li from the Lengshuihe

301   Nature Reserve in Jinsha Co., Guizhou Prov., China. Six males: CIBjs20150803001, CIBjs20150803003,

302   CIBjs20150803004, CIBjs20150803005 collected on August 3, 2015 and CIBjs20150804002 on August 4,

303   2015; eight females: CIBjs20150803006, CIBjs20150803007, CIBjs20150803008 collected on August 3, 2015,

304   CIBjs20150804001, CIBjs20150804003, CIBjs20150804004, CIBjs20150804005 on August 4, 2015 and

305   CIBjs20150808020 on August 8, 2015. Seven specimens collected by S.Z. Li and J. C. Lv from Meitan Co. of

306   Guizhou Prov., China. Three males: CIBGYU20130917004, CIBGYU20130917005 and

307   CIBGYU20130917007 collected by J.C. Lv on September 17, 2013; four females: CIBGYU20130917001,

308   CIBGYU20130917002 and CIBGYU20130917003 by S.Z. Li on September 17, 2013, CIBGYU20130917006

309   by J.C. Lv on September 17, 2013. Three females: GYU20130921001, CIBGYU20130921002 and

310   CIBGYU20130921003 collected by S.Z. Li on September 21, 2013. Ten tadpoles (CIBjs20171014001-

311   CIBjs20171014010) collected by S.Z. Li on October 10, 2017.

312   **Diagnosis**

313   *Odorrana kweichowensis* **sp. nov.** is assigned to genus *Odorrana* based upon molecular phylogenetic

314   analyses and the following morphological characters: dorsum is green; tips of digits dilated, tapering, disks

315   with circum-marginal grooves, and vertical diameter longer than horizontal diameter in the disks;

316   supernumerary tubercle below the base of fingers III and IV; feet fully webbed to disks, without tarsal fold; the

317   first finger thick and nuptial pad distinct.

318   *Odorrana kweichowensis* **sp. nov.** could be distinguished from its congeners by a combination of the

319   following characters: (1) having smaller body size in males (snout-vent length < 43.3 mm vs. snout-vent length >

320   48 mm in many other species); (2) head longer than wider; (3) dorsolateral folds absent; (4) tympanum of

321   males large and distinct, tympanum diameter in males twice as long as width of distal phalanx of finger III; (5)

322   two metacarpal tubercles; (6) relative finger lengths: II < I < IV < III; (7) tibiotarsal articulation reaching to the

323   level between eye to nostril when leg stretched forward; (8) disks on digits with circum-marginal grooves; (9)

324   toes fully webbed to disks; (10) the first subarticular tubercle on fingers weak; (11) having white pectoral

325   spinules, paired subgular vocal sacs located at corners of throat, light yellow nuptial pad on the first finger in

326   males.

327   **Description of holotype**

328   Head longer than wide (HDL/HDW = 1.08); top of head flat; snout obtusely rounded in dorsal view

329   (SL/HDL = 0.41), rounded in profile, projecting beyond lower jaw; eye large and convex, ED 0.83 times of SL;

330   IND = 5.1 mm, larger than IOD = 3.2 mm; NED = 3.4 mm larger than NSD = 2.9 mm; tympanum circular,

331   large and distinct, twice as long as width of distal phalanx of finger III (TYD/FDW = 2.84); vomerine teeth on

332   well-developed ridges; tongue deeply notched posteriorly; paired gular pouches at corners of throat. Forelimbs

333   moderately robust (LW/SVL = 0.11); lower arm and hand beyond a half of body length (LAL/SVL = 0.53); the

334   first finger slightly longer than the second; finger tips on I–IV dilated to wide, tapering, disks with circum-

335   marginal grooves; nuptial pad on the inner of first finger from base to subarticular tubercles; subarticular

336   tubercles relatively prominent; inner metacarpal tubercle oblong and outer metacarpal tubercle indistinct; no

337 finger webbing.

338   Hindlimbs long; tibio-tarsal articulation reaching to the level between eye to nostril when hindlimb

339 adpressed along the side of body; heels overlapping when hindlimbs positioned at right angle to body; singular

340 longer than thigh length; FL 0.85 times SVL; toes slender, relative toe lengths: I < II < III < V < IV; toes

341 tapering dilated, disks with circum-marginal grooves; feet fully webbed to disks; web becoming narrower and

342 continuing to the disks as lateral fringe on toes II, III and IV; outer metatarsal tubercle absent; inner metatarsal

343 tubercle present.

344   Skin shagreened, a number of pustules scattered on dorsum of trunk and flanks; several irregular tubercles

345 scattered on flanks; dorsolateral folds absent; two large glands on the place between below tympanum and

346 above arm; white spinules on throat, chest, fore abdomen, inner side of forearms and upper and lower lips.

347 **Coloration in life**

348   In life, dorsum with bright green network ; large black spots in the center on dorsum, continuing onto

349 dorsal portion of legs to form dark counter-point to bright green irregular bands; flank light yellow with

350 several black spots; tympanum brown-black; upper and lower lip with vertical black bars; arms brown with

351 black transverse bands, thighs with five brown bands and tibias with six (Fig. 6).

352 **Color in preservative**

353   On dorsum color fades to dark olive with dark brown blotches (Fig. 5A), upper and lower lips marbled

354 brown-black on dirty white; venter variable from white to light yellow; underside of limbs yellowish with

355 white (Fig. 5B); pinkish in the inner side of forearm and thighs; there is a white spot between front corner of

356 eyes; nuptial pad fades to white (Fig. 5).

357 **Variation**

358   Basic statistics for measurements were presented in Table S6. All male specimens were similar in

359   morphology and color pattern, but different from females. SVL in females approximately 1.8 times that in

360   males (SVL mean 73.6 mm, range 62.4–81.1 mm in females, SVL mean 41.0 mm, range 36.2–43.3 mm in

361   males); the ratios of ED, IND, IFE, LAL, TYD and LW to SVL of males are significantly higher than that of

362   females, but the ratios of TW to SVL of males is lower than that of females (Table 1). In some adult females,

363   the black transverse bands on limbs are not obvious (Fig. 6C). In some adult females, chest, chin and ventral

364   surface of limbs scattered with continued light-brown spots (Fig. 7A). Some adult females have black cloud

365   stripe with irregular borders on the ventral side (Fig. 7B), but some don't have (Fig. 6D). In some adult

366   females, dorsum uniform brown with little green impression (Fig. 7C) and some adult females have dorsum

367   uniform brown lacking green impression (Fig. 7D).

368   **Skull description**

369   The skull morphology of the five scanned specimens were almost identical, and thus, only one

370   representative (voucher number: CIBjs20150803001) was presented (Fig. 8) and described as following: skull

371   flat, maxillary teeth well developed, vomerine teeth present; mandible without teeth; nasals large, widely

372   separated from each other, nasals disconnected with the sphenethmoid; sphenethmoid tubular, forms the

373   anterolateral walls of the braincase; frontoparietal roof the braincase and wider posteriorly than anteriorly;

374   prootic large and connect with the exoccipital; a pair of exoccipitals situated the end of the brain; palatines

375   arcuate and long, behind the prevomers; a pair of prevomers obliquely lie anterior to the palatines, vomerine

376   teeth on the prevomers indistinct; parasphenoid in sword shape, supports the braincase ventrally, connects with

377   palatines; a pair of squamosals on the dorsolateral side of the prootic, each squamosal consists of three rami:

378   the zygomatic ramus, the otic ramus, and the ventral ramus; zygomatic ramus pointing to the orbit, otic ramus

379   shorted, ventral ramus outboard and covered the posterior of pterygoid; a pair of pterygoids, outside of the

380   ventral surface of the squamosal, anterior ramus is the longest, center is inward and leading edge extends to the

381   orbit, medial ramus shorted and attached to the anterior lateral part of the prootic, posterior ramus edge-on and

382   extend to maxillary; a pair of columellaes situated ventral to the crista parotica (Fig. 8).

383   **Tadpole description**

384   Body and tail yellowish-brown (Fig. 9A), at Gosner's stage 28-29, TOL 31.6–36.2 mm, SVL 12.1–14.5

385   mm, other measurements of tadpoles shown in Table S4. Tail 1.5–1.8 times as long as body; TH 25–30% of

386   TL in the 28th stage and 23–28.5% in the 29th stage; BW 45.8% of SVL in the 28th stage and 46.8% in the

387   29th stage; BH 31.4% of SVL in the 28th stages and 34.9% in the 29th stage; tail fins lightly colored, tail

388   muscles with small black spots; tail depth greater than body depth, dorsal fin arising behind the origin of the

389   tail; maximum depth near mid-length, tip of tail blunt; nostril near snout, eyes positioned dorsally (Fig. 9A);

390   spiracle on the middle left of body (Fig. 9B); keratodont formula I: 3-3/ III: 1-1 (Fig. 9C); both upper and

391   lower lips with labial papillae (Fig. 9C); some additional tubercles at the angles of the mouth, usually with

392   small keratodonts (Fig. 9C).

393   **Sexual dimorphism**

394   SVL in females approximately 1.8 times that in males (SVL mean 73.6 mm, range 62.4–81.1 mm in

395   females; SVL mean 41.0 mm, range 36.2–43.3 mm in males); paired subgular vocal sacs located at corners of

396   throat, pinkish lineae musculinae on dorsal side, nuptial pad light yellow on the inner of first finger from base

397   to subarticular tubercles in males (Figs. 5, 6).

398   **Comparisons**

399   *Odorrana kweichowensis* **sp. nov.** differs from *O. amamiensis*, *O. andersonii*, *O. bacboensis*, *O.*

400   *cangyuanensis, O. chapaensis, O. geminata, O. graham, O. hmongorum, O. ishikawae, O. jingdongensis, O.*

401   *junlianensis, O. kuangwuensis, O. macrotympana, O. margaretae, O. mawphlangensis, O. mutschmanni*, and

402   *O. wuchuanensis*, by having smaller body size (SVL < 43.30 mm in males in the new species vs. SVL > 48

403   mm in males in other species).

404   *Odorrana kweichowensis* **sp. nov.** differs from *O. absita, O. aureola, O. banaorum, O. bolavensis, O.*

405   *exiliversabilis, O. gigatympana, O. graminea, O. hosii, O. heatwolei, O. indeprensa, O. khalam, O. leporipes,*

406   *O. livida, O. monjerai, O. narina, O.nasica, O. nasuta, O. orba, O. supranarina, O. tormota, O. trankieni, O.*

407   *utsunomiyaorum, O. versabilis, O. yentuensis* and *O. zhaoi*, by lacking dorsolateral folds (vs. present in other

408   species).

409   *Odorrana kweichowensis* **sp. nov.** differs from *O. fengkaiensis* by tibiotarsal articulation reaching the

410   level between eye to nostril when leg stretched forward (vs. reaching the level below nostril in the latter); from

411   *O. rotodoar* by tibiotarsal articulation reaching the level between eye to nostril when leg stretched forward (vs.

412   reaching the level beyond eye in the latter); from *O. lipuensis* (vocal sacs absence in males) and *O. hainanensis*

413   (paired internal vocal sacs in males) by paired external vocal sacs located at corners of throat; from *O.*

414   *chloronota* by having head longer than wide (vs. head wider than long in the latter); from *O. morafkai* and *O.*

415   *sinica* by having white pectoral spinules in mature males (vs. absence in the latter); from *O. arunachalensis*, by

416   the relative finger lengths I < II < IV < III (vs. II < I < IV < III in the latter).

417   Within the *O. schmackeri* group (*O. anlungensis, O. hejiangensis, O. lungshengensis, O. nanjiangensis, O.*

418   *swinhoana, O. tianmuii, O. tiannanensis, O. yizhangensis, O. huanggangensis* and *O. schmackeri*), *Odorrana*

419   *kweichowensis* **sp. nov.** differs from *O. anlungensis* by having white pectoral spinules in mature males (vs.

420   absence in the latter), tibiotarsal articulation reaching the level between eye to nostril when leg stretched

421   forward (vs. reaching the nostril in the latter), web becoming narrower and continuing to the disks of toe IV

422   (vs. web continuing to the subarticular tubercles of toe IV in the latter); from *O. hejiangensis* by the disks of

423   fingers with circum-marginal grooves (vs. without circum-marginal grooves on finger I in the latter), two

424   metacarpal tubercles (vs. three metacarpal tubercles in the latter); from *O. lungshengensis* by the body size of

425   males with maximum SVL 43.3 mm (vs. SVL 50.0–59.6 mm of males in the latter), tympanum large and

426   distinct, and beyond twice as long as width of distal phalanx of finger III (vs. tympanum as long as width of

427   distal phalanx of finger III in the latter), two metacarpal tubercles (vs. without outer metacarpal tubercle in the

428   latter), tibiotarsal articulation reaching the level between eye to nostril when leg stretched forward (vs.

429   reaching the tip of snout in the latter); from *O. nanjiangensis* by the body size in males with maximum SVL

430   43.3 mm (vs. SVL 59.5–66.8 mm in males in the latter), two metacarpal tubercles (vs. without outer

431   metacarpal tubercle in the latter), tibiotarsal articulation reaching the level between eye to nostril when leg

432   stretched forward (vs. reaching the tip of snout in the latter); from *O. swinhoana* by the body size in males with

433   maximum SVL 43.3 mm (vs. SVL 47.7–71.6 mm in males in the latter), tibiotarsal articulation reaching the

434   level between eye to nostril when leg stretched forward (vs. reaching the tip of snout); from *O. tianmuii* by the

435   relative finger lengths II < I < IV < III (vs. I < II < IV < III in the latter), two metacarpal tubercles (vs. three

436   outer metacarpal tubercles in the latter); *O. tiannanensis* by the body size in males with maximum SVL 43.3

437   mm (vs. SVL 52.5–53.5 mm in males in the latter), tibiotarsal articulation reaching the level between eye to

438   nostril when leg stretched forward (vs. reaching the level beyond the tip of snout in the latter); *O. yizhangensis*

439   by the body size in males with maximum SVL 43.3 mm (vs. SVL 47.3–54.0 mm in males in the latter), two

440   metacarpal tubercles (vs. without outer metacarpal tubercle), tibiotarsal articulation reaching the level between

441   eye to nostril when leg stretched forward (vs. reaching the tip of snout in the latter); from *O. huanggangensis*

442   by the relative finger lengths II < I < IV < III (vs. I < II < IV < III in the latter), tibiotarsal articulation reaching

443   the level between eye to nostril when leg stretched forward (vs. reaching the nostril in the latter); from *O.*

444   *schmackeri*, by the first subarticular tubercle on fingers and supernumerary tubercles being weak (vs. being

445   outstanding in the latter; Fig. 5E, F), and having two outer metacarpal tubercles (vs. outer metacarpal tubercle

446   being indistinct in the latter; Fig. 5H, G).

447   *Odorrana kweichowensis* **sp. nov.** differs from *O. schmackeri* by having significantly lower ratios of HDL

448   and SL to SVL in males, having significantly higher ratios of THL, TL and FL to SVL in males, and having

449   significantly higher ratios of IND, THL, TL and FL to SVL in females (all *p*-values < 0.05; Table 1; Table S6);

450   from *O. huanggangensis* by having significantly lower ratios of NSL and IAE to SVL in males, having

451   significantly higher ratios of IND, LAL, THL and TFL to SVL in males, and having significantly higher ratios

452   of LAL, THL, TL and FL to SVL in females (all *p*-values < 0.05; Table 1; Table S6).

453   **Ecology**

454   To present, *Odorrana kweichowensis* sp. nov. has been found in three localities: Lengshuihe Nature

455   Reserve (27°34'–27°26' N, 105°57'–106°04' E) in Jinsha Co., Meitan Co. (27°39'–27°42' N, 107°33'–107°35' E)

456   and Zheng'an Co. (28°09'–28°20' N, 107°30'–107°38' E) in Guizhou Prov. of China. Geographical distances

457   between these localities were from 89 km to 173 km. Population from the Lengshuihe Nature Reserve

458   inhabited broad streams, and near the riparian areas, surrounded by evergreen broadleaved forests (Fig. 10A).

459   Populations from Meitan Co. and Zheng'an Co. inhabited broad slow-flowing rivers surrounded by paddy field

460   (Fig. 10B and C). All of the localities were at elevations 717-766 m. All adult individuals that we found appear

461   on the stones in the streams at night (07:30-12:00 pm) with water pH 6.8-7.1 and water temperature 15-23 °C.

462   Tadpoles could be found at daytime and night. Amplexed individuals could be found in the streams in the type

463   locality (Fig. 10A). Three sympatric amphibian species *Fejervarya multistriata*, *Rana zhenhaiensis* and

464   *Polypedates megacephalus* were found in Meitan Co. and Zheng'an Co., but only one sympatric amphibian

465   species *Amolops chunganensis* was found in the Lengshuihe Nature Reserve in the type locality.

466   **Etymology**

467   The specific epithet "*kweichowensis*" refers to the distribution of this species, Guizhou Prov., China. The

468   "kweichow" is an old spelling and a transliteration for "Guizhou". We propose the common English name

469   "Guizhou Odorous Frog" for this species.

470

471   **DISCUSSION**

472   Morphological similarity of related species in the genus *Odorrana* challenges classifications in the group

473   (Fei et al., 2009; Chen et al., 2013). Integrative taxonomy with multiple evidences, such as genetic divergence,

474   ecological discrepancy, morphometric differences, and so on, turns out to be quite effective, and have already

475   become the main trend. In this study, based on molecular phylogenetic analyses and morphological

476   comparisons, a new species, *Odorrana kweichowensis* sp. nov., was described from mountain streams in

477   Guizhou Prov., China. Phylogenetic analyses based on mitochondrial DNA suggested that the new species

478   belonged to *Odorrana* but was significantly separated from its congeners. Genetic distance based on 16S

479   rRNA gene between it and its sister species *O. schmackeri* was mean 0.027, matching the level about

480   interspecific divergences in amphibians (0.01-0.17; Vences et al. 2005) and being much higher than that

481   between many sister species (of which, most species have been completely recognized as valid species) in

482   *Odorrana*. Moreover, on nuclear protein-coding genes which presented much lower evolutionary rates, the

483   new species was still indicated to occupy an independent lineage separated from other species. These results

484   confirmed restricted even absent gene flow between the new species and its related species. Finally, the new

485   species was different from its congeners on a lot of morphological characters. At all, multiple evidences

486   supported the validity of the new species.

487   In this study, *Odorrana kweichowensis* sp. nov. was found in three localities in the northwestern part of

488   Guizhou Prov. of China (Fig. 1). In Li et al. (2015), the new species was only found in two localities in

489   Guizhou Prov.: one was the type locality (Lengshuihe Reserve in Jinsha Co.) of it, and another was the

490   Suiyang Co. closely adjoining Meitan Co. and Zheng'an Co. included in this study. Obviously, it could be

491   speculated that the new species was at least distributed in the northwestern part of Guizhou Prov. of China (Fig.

492   1). Unfortunately, it was still not sure whether the new species was conspecific with *Odorrana* sp1 recognized

493   by Zhu et al. (2016) in absence of corresponding specimens and sequences. If they were conspecific, the new

494   species should be distributed in a larger range from northern Guizhou Prov. to southern Guangxi Prov., China.

495   In addition, it could not infer whether the populations in northern Thailand and northern Vietnam reported as *O.*

496   *schmackeri* (Chan-ard, 2003; Orlov et al., 2002; Nguyen et al., 2005; Fig. 1) was conspecific with the new

497   species, because there was also no molecular data and detailed morphological descriptions of them. As note,

498   according to previous studies (Li et al., 2015; Zhu, 2016) and our results, the new species was possibly

499   sympatric with Clade D (*Odorrana* sp2) recognized by Zhu (2016) and *O. hejiangensis* in a narrow area along

500   the boundary between northern Guizhou Prov. and Sichuan Prov. and Chongqing City, China (Fig. 1), though

501   in the localities investigated in this study, we did not find the latter two species in the microhabitats of the new

502   species.

503

504   **CONCLUSION**

505   We described a new species of the odorous frog genus *Odorrana* (Amphibia, Anura, Ranidae) from

506   Guizhou Prov. of China, and provide evidence for its phylogenetic allocations. *Odorrana kweichowensis* sp.

507   nov. was only known from a narrow range in the northwestern part of Guizhou Prov. of China, and occurred

508   from mountain streams at mid and low elevations similar to most odorous frogs. In our fieldwork, the new

509   species was found to be seriously threatened by local villager and construction of dams and roads. Thus,

510   further more detailed investigations on the species are urgent to ascertain its distributional range and

511   population status. With our description we contributed to a better knowledge of the diversity of the genus

512   *Odorrana* in the southwestern China, and thus suggested that more comprehensive phylogeographic studies

513   would highlight radiation patterns of the group.

514

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518

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The results of one-way ANOVA with *p*-values for morphometric comparisons between *Odorrana kweichowensis* sp. nov., *O. schmackeri* and *O. huanggangensis*.

OK, *O. kweichowensis* sp. nov.; *OS*, *O. schmackeri*; *OH*, *O. huanggangensis*. Significance level: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. Abbreviations for the morphometric characters refer to Methods section.

	Males of *OK* vs. Females of *OK*	In males		In females	
		OK vs. *OS*	*OK* vs. *OH*	*OK* vs. *OS*	*OK* vs. *OH*
SVL	0.0001***	0.3160	0.2370	0.0810	0.4520
HDL	0.5560	0.0030**	0.5740	0.0900	0.0540
HDW	0.6240	0.2100	0.7650	0.0560	0.9000
SL	0.9840	0.0130*	0.1130	0.0740	0.9860
ED	0.0001***	0.4730	0.2510	0.1560	0.9130
IOD	0.1470	0.0910	0.2370	0.4520	0.2880
IND	0.0210*	0.5770	0.0330*	0.0290*	0.8340
NED	0.1470	0.2790	0.8480	0.4940	0.8860
NSD	0.8830	0.0980	0.0430*	0.3060	0.0310*
IFE	0.0001***	0.2140	0.3870	0.0740	0.6930
IAE	0.0001***	0.0490*	0.0310*	0.2320	0.2060
TYD	0.0001***	0.1960	0.2980	0.9260	0.1040
LAL	0.3340	0.0950	0.0310*	0.0320*	0.0000***
LW	0.0040**	0.1090	0.1390	0.3510	0.6420
THL	0.7560	0.0110*	0.0240*	0.0270*	0.0000*
TL	0.0920	0.0170*	0.1970	0.0420*	0.0040**
TW	0.0220*	0.0840	0.1430	0.1500	0.0160*
TFL	0.9930	0.0540	0.0290*	0.0500	0.0390
FL	0.2690	0.0170*	0.2410	0.0270	0.0050**
FDW	0.3400	0.0840	0.5660	0.9480	0.5520
DPW	0.5490	0.1040	0.6290	0.4520	0.6510

Figure 1

Sampling localities in this study.

Localities 1-8 were all in China. 1, Jinsha County (Co.), Guizhou Province (Prov.); 2, Zheng'an Co., Guizhou Prov.; 3, Meitan Co., Guizhou Prov.; 4, Leishan Co., Guizhou Prov.; 5, Jiangkou Co., Guizhou Prov.; 6, Sangzhi Co., Hunan Prov.; 7, Changyang Co., Hubei Prov.; 8, Ruyuan Co., Guangdong Prov.. According to literatures and results in this study, the distributional range of each related species was surrounded by dotted lines on the map. *Odorrana* sp1 and *Odorrana* sp2 were proposed in Zhu (2016); *Odorrana* sp3 was corresponding to the third cryptic species (Clade F) proposed in Li et al. (2015). Different species were denoted as different colors and shapes.
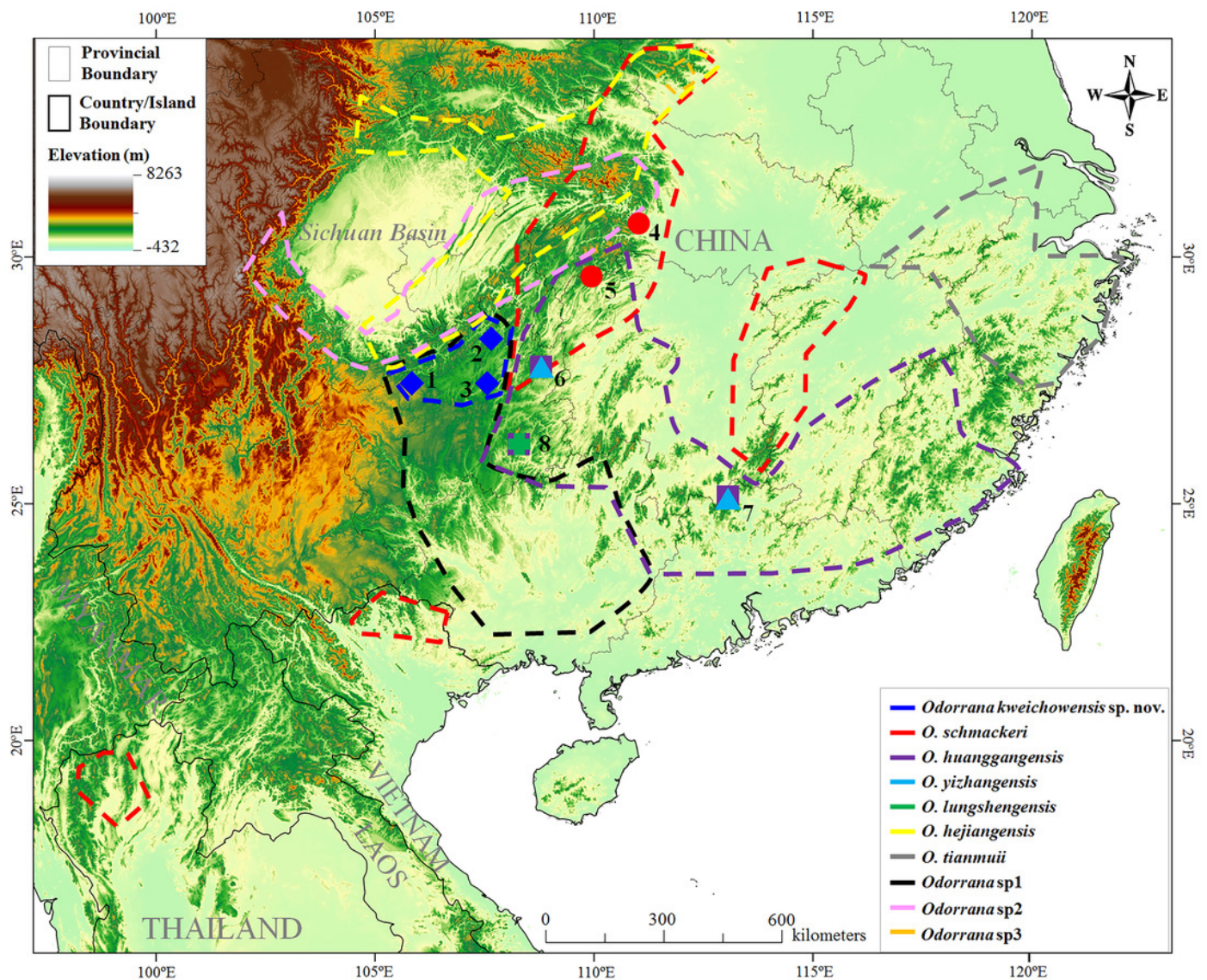
Figure 2

Phylogenetic relationships of *Odorrana kweichowensis* sp. nov. and its congeners.

(A) Maximum likelihood (ML) tree reconstructed based on the three genes (12S + 16S + ND2) concatenated dataset. (B) Phylogenetic network of *O. kweichowensis* sp. nov. and its related species reconstructed by the software Splittree based on ND2 gene sequences. In ML tree, bootstrap supports (BS) from ML analyses/Bayesian posterior probabilities (BPP) from BI analyses were noted beside nodes. In Splittree network, numbers on branches are BS. *Odorrana* sp2 was proposed in Zhu (2016); *Odorrana* sp3 was corresponding to the third cryptic species (Clade F) in Li et al. (2015). Different related species of *Odorrana kweichowensis* sp. nov. were denoted as different colors.
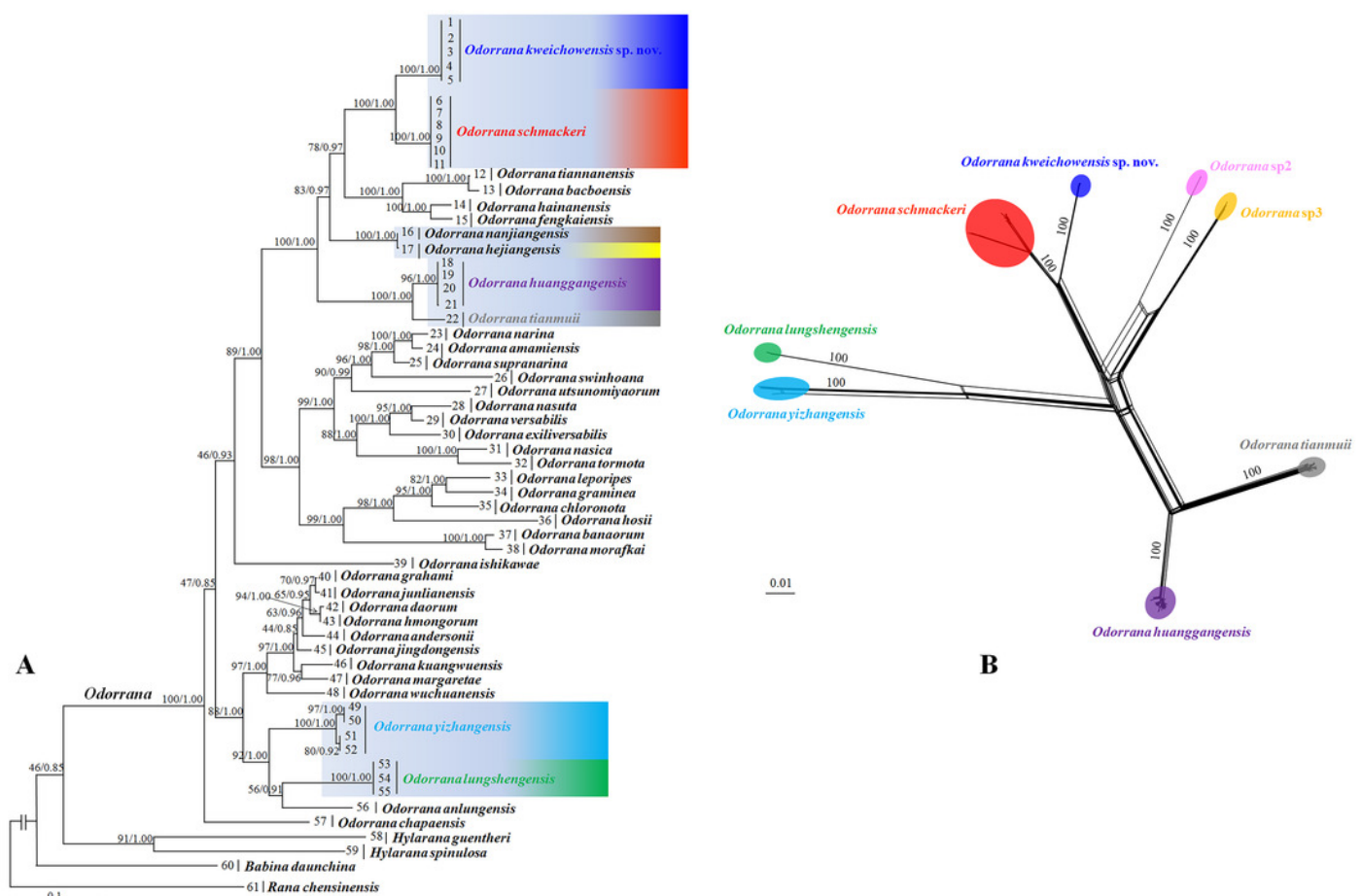
Figure 3

Haplotype networks of *O. kweichowensis* sp. nov. and its related species constructed based on the nuclear gene sequences.

(A) DOLK gene. (B) KCNF gene. Different species were denoted as different colors.
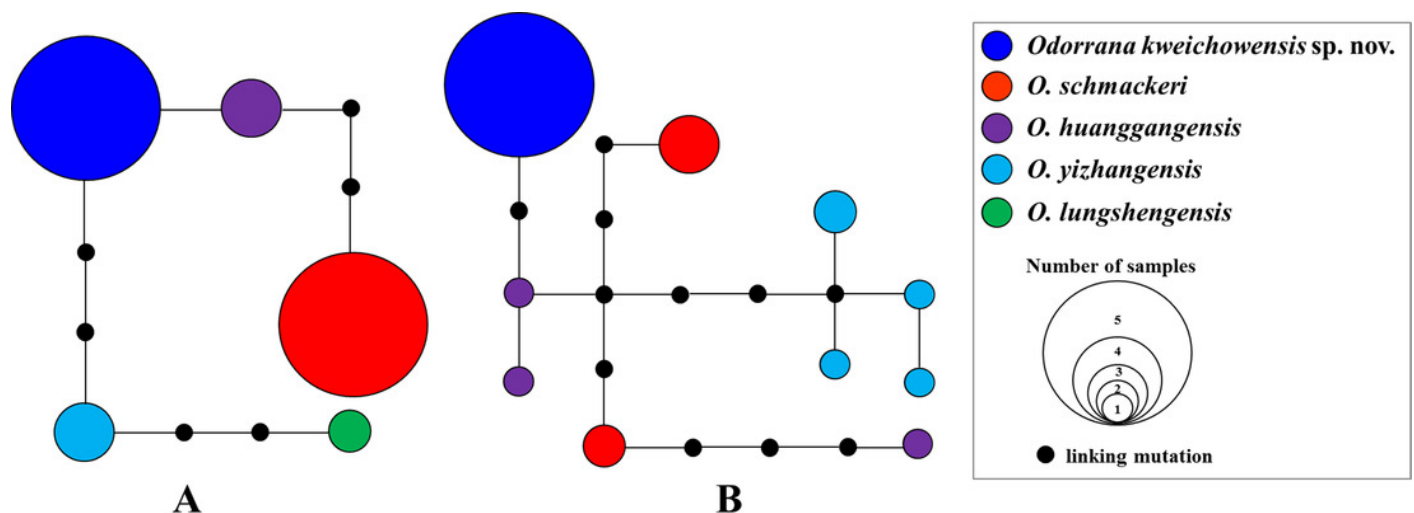
Figure 4

Plots of principal component analyses of *Odorrana kweichowensis* sp. nov., *O. schmackeri* and *O. huanggangnesis.*

(A) Males. (B) Females. PC1, the first principal component; PC2, the second principal component. Different species were denoted as different colors and shapes.
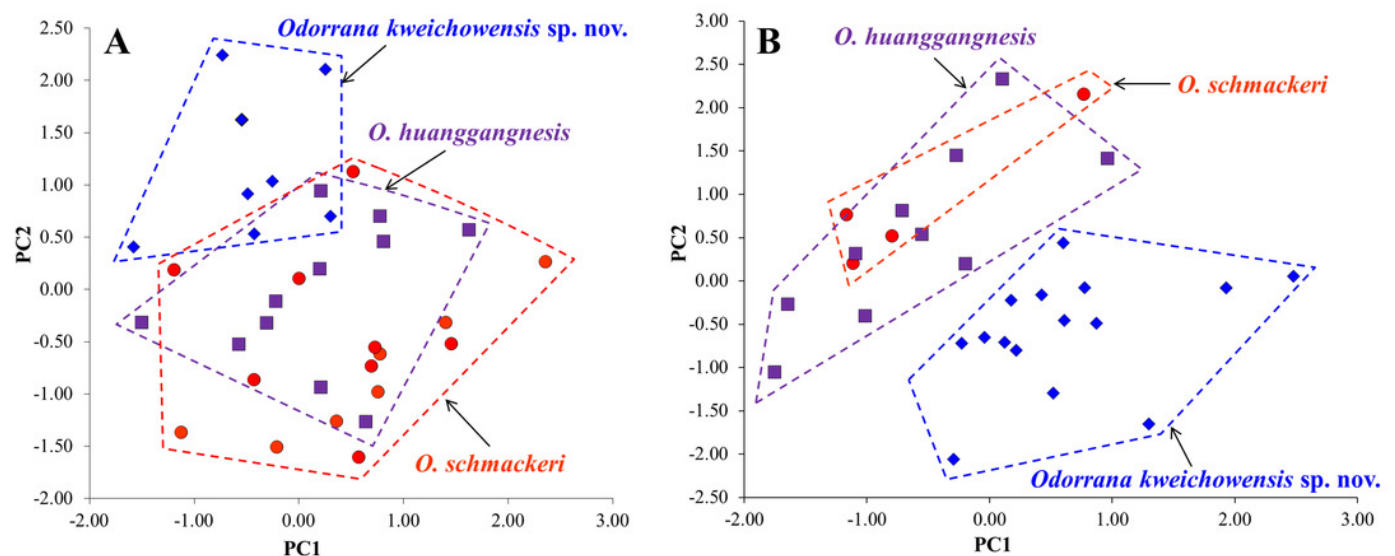
Figure 5

Comparisons of the holotype (voucher number: CIBjs20150803002) of *Odorrana kweichowensis* sp. nov. and one male specimen (voucher number: CIBsz2012062003) of *O. schmackeri.*

(A) and (B), dorsal view and ventral view of specimen CIBjs20150803002, respectively. (C) and (D), dorsal view and ventral view of specimen CIBsz2012062003, respectively. (E) Ventral view of hand of specimens CIBjs20150803002. (F) Ventral view of hand of specimen CIBsz2012062003. (G) Ventral view of foot of specimen CIBjs20150803002. (H) Ventral view of foot of specimen CIBsz2012062003. Photographs by S.Z. Li.

Note: Auto Gamma Correction was used for the image. This only affects the reviewing manuscript. See original source image if needed for review.
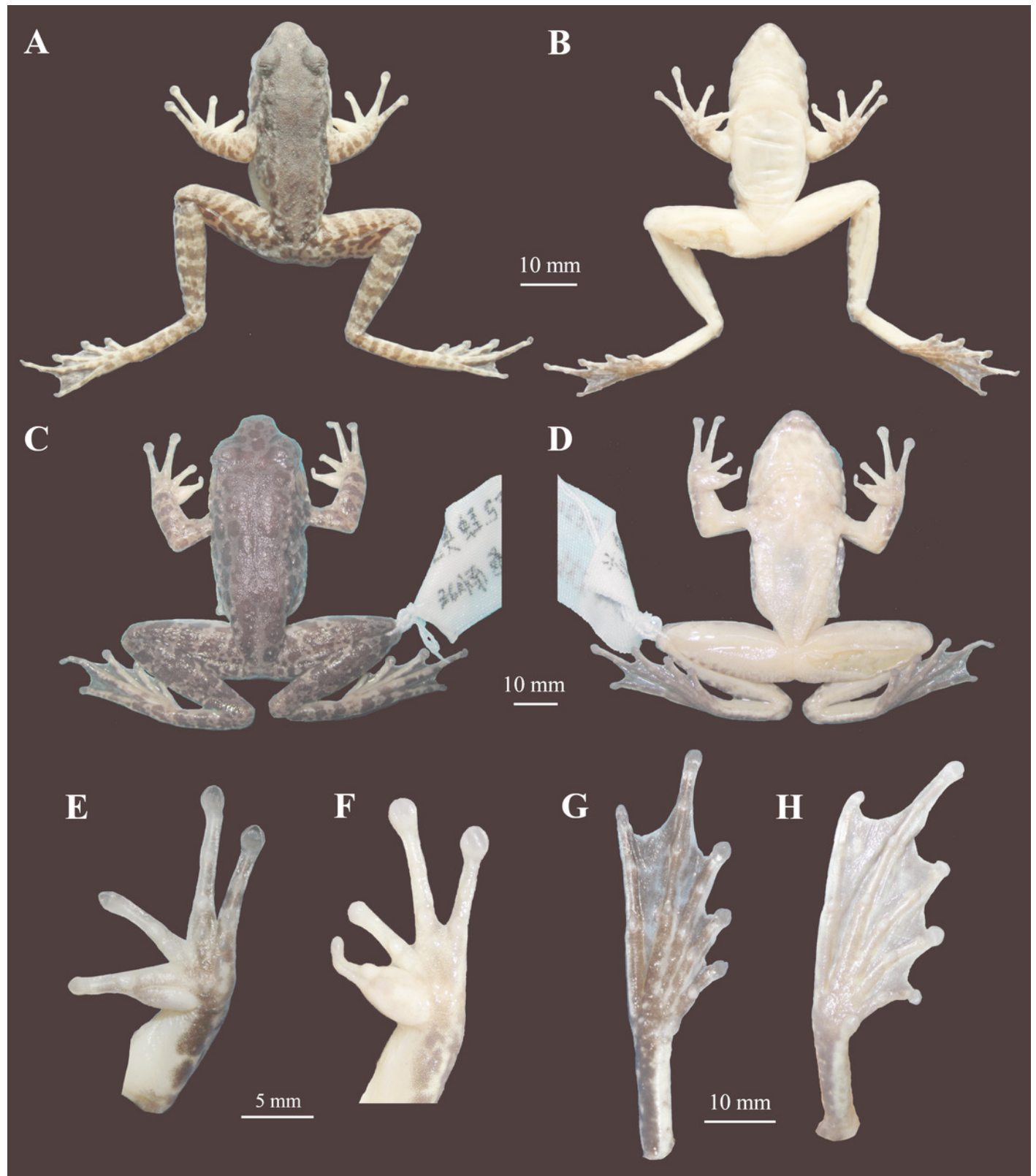
Figure 6

Living *Odorrana kweichowensis* sp. nov. from its type locality, Lengshihe Nature Reserve in Jinsha County, Guizhou Province, China.

(A) and (B), dorsolateral view and ventral view of an adult male (voucher number: CIBjs20150803002), respectively. (C) and (D), dorsolateral view and ventral view of an adult female (voucher number: CIBjs20150803006), respectively. Photographs by S.Z. Li.
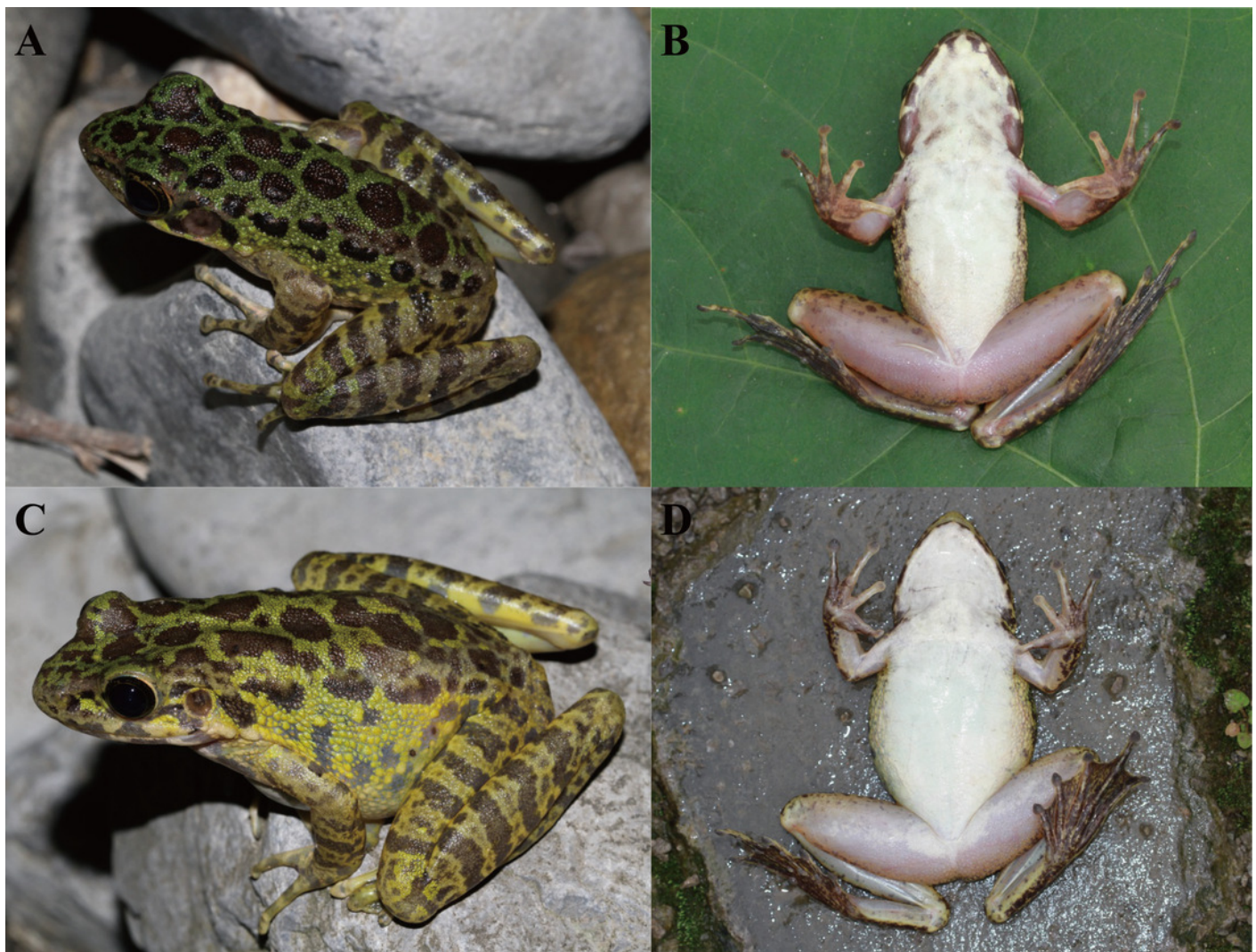
Figure 7

Color variations in *Odorrana kweichowensis* sp. nov..

(A) and (B), dorsolateral view and ventral view of an adult female from Jinsha County, Guizhou Province, China, respectively. (C) Dorsolateral view of a female from Meitan County, Guizhou Province, China. (D) Dorsolateral view of a female from Zheng'an County, Guizhou Province, China. Photographs by S.Z. Li.
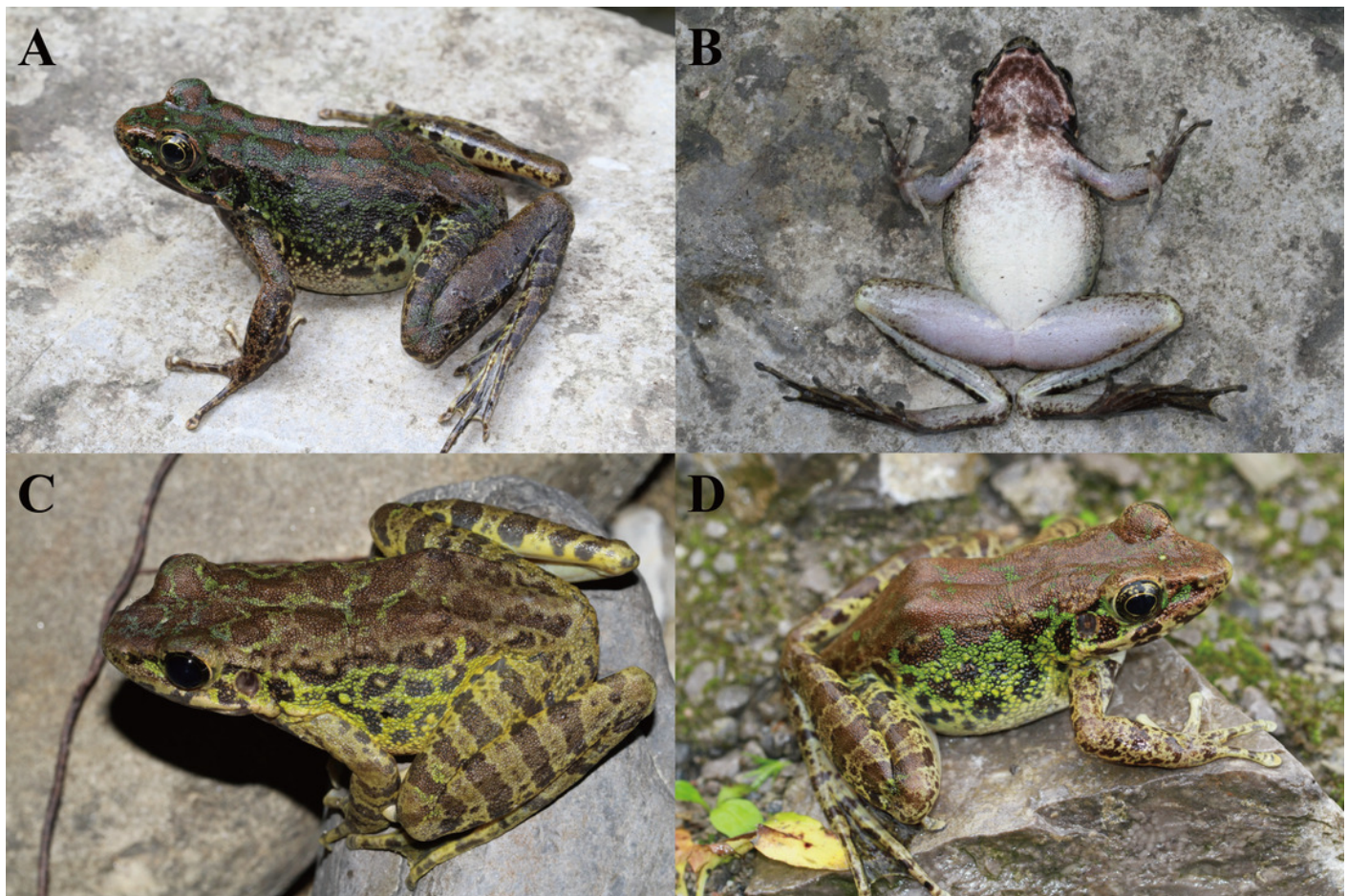
Figure 8

Skull of *Odorrana kweichowensis* sp. nov..

(A) Dorsal view. (B) Ventral view. 1, maxillary; 2, nasal; 3, frontoparietal; 4, pterygoid; 5, squamosal; 6, exoccipital; 7, prootic; 8, maxillary teeth; 9, prevomer; 10, palatine; 11, mandible; 12, sphenethmoid; 13, parasphenoid; 14, columella. Drawings by S.Z. Li.
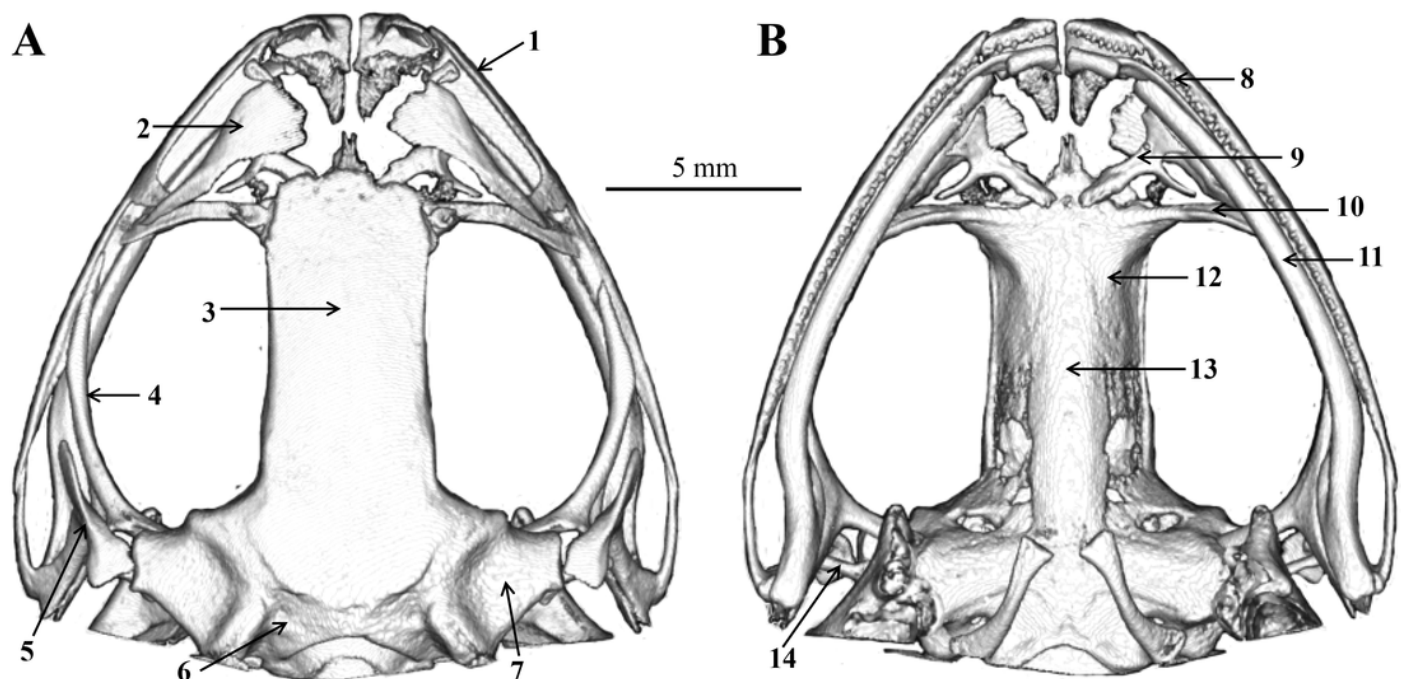
Figure 9

Tadpole of *Odorrana kweichowensis* sp. nov.

(A) Dorsolateral view of specimen CIBJS20171014001 in life. (B) Dorsal view of specimen CIBJS20171014001 in preservative. (C) Structure of the mouth of specimen CIBJS20171014001. 1, spiracle; 2, upper keratodonts; 3, lower keratodonts; 4, labial papillae on upper lips; 5, labial papillae on lower lips; 6, additional tubercles at the angles of mouth. Photographs by S.Z. Li.

Note: Auto Gamma Correction was used for the image. This only affects the reviewing manuscript. See original source image if needed for review.
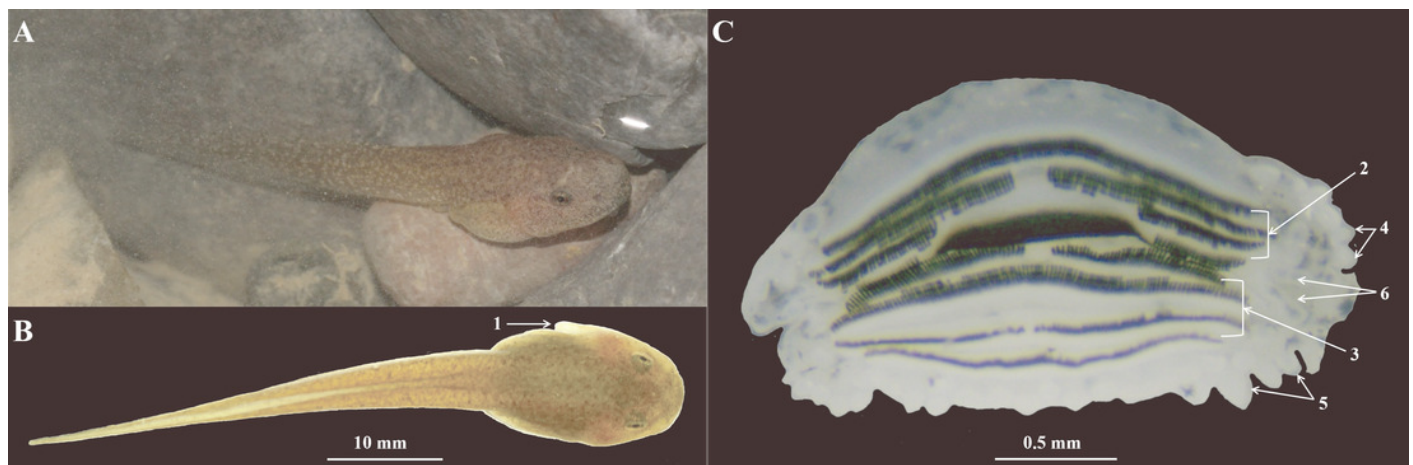
Figure 10

Habitats of *Odorrana kweichowensis* sp. nov..

(A) Habitats in the type locality, Lengshuihe Nature Reserve, Jinsha County, Guizhou Province, China; *insert* is the photo for one pair of amplexed male (smaller) and female (larger) found on the stone in the stream. (B) Habitats in Xieba Town, Zheng'an County, Guizhou Province, China. (C) Habitats in Shilian Town, Meitan County, Guizhou Province, China. Photographs by S.Z. Li.